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Nephrotoxic and Genotoxic *N*-Acetyl-*S*-dichlorovinyl-*L*-cysteine Is a Urinary Metabolite after Occupational 1,1,2-Trichloroethene Exposure in Humans: Implications for the Risk of Trichloroethene Exposure

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Excretion of mercapturic acids in the urine is indicative of the formation of electrophiles in the metabolism of xenobiotics. The determination of these mercapturic acids thus may be a useful method to estimate the exposure. We identified the nephrotoxic and mutagenic mercapturic acids *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine in the urine of workers exposed to 1,1,2-trichloroethene. A method to quantify these mercapturic acids by gas chromatography–mass spectrometry-selected ion monitoring was developed and appreciable amounts (2.8–3.8 μmole/L were found in human urine samples. Because deacetylation determines notably the amount of the excreted mercapturic acids, the formation of the resulting cysteine *S*-conjugates was comparably measured in subcellular fractions of rodent and human kidneys; significant species differences in acylase activity were found. The formation of mutagenic and nephrotoxic metabolites during 1,1,2-trichloroethene metabolism mandates a revision of the risk assessment of trichloroethene exposure.

Introduction

1,1,2-Trichloroethene (trichloroethene) is an important industrial chemical which is widely used because of its favorable solvent characteristics, chemical stability, and relatively low acute toxicity. The carcinogenicity of trichloroethene has been extensively debated over the past 15 years. High doses of trichloroethene increase the rate of hepatocellular carcinomas in B6C3F$_1$ mice and induce renal tubular adenocarcinoma in male Fischer F344 rats (*1*). Bioactivation reactions are likely responsible for trichloroethene carcinogenicity. In both rats and mice, trichloroethene is metabolized by two pathways, oxidation by cytochrome P-450 and conjugation with glutathione by glutathione-*S*-transferases (Fig. 1). Glutathione (GSH) conjugation to *S*-(1,2-dichlorovinyl)glutathione (DCVG) might initiate trichloroethene nephrocarcinogenicity. DCVG is cytotoxic, mutagenic, and nephrotoxic. The formed DCVG is cleaved by the enzymes of mercapturic acid formation to *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC). After accumulation in the kidney by active transport mechanisms, DCVC is a substrate for renal cysteine conjugate β-lyase and is cleaved to yield pyruvate, ammonia, and the electrophile chlorothioketene (*2*). Evidence for the occurrence of this pathway in trichloroethene metabolism *in vivo* has been obtained by the identification in rat urine of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine, the excretory product of the processing of DCVG by the enzymes of mercapturic acid formation (*3,4*). DCVG was also identified as a biliary metabolite of trichloroethene in rats *in vivo* and as the product of trichloroethene biotransformation in rat liver microsomes in the presence of GSH.

Trichloroacetic acid, a metabolite formed by oxidative metabolism, which is the major pathway of trichloroethene metabolism *in vivo*, induced peroxisome proliferation selectively in mouse liver, a mechanism that may explain the hepatocarcinogenicity of trichloroethene in mice (*5*). This process may not be relevant for human carcinogenic risk of trichloroethene exposure at lower doses, as oxidative metabolism does not result in mutagenic products. The experiments reported here demon-

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Table 1. Excretion of trichloroacetic acid and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine in the urine of four workers after exposure (by inhalation or skin contact) to 1,1,2-trichloroethene.

Worker number	*N*-acetyl-*S*-dichlorovinyl-l-cysteine, μmole/L[a]	Trichloroacetic acid, μmole/L	Trichloroacetic acid/mercapturic acid
1	2.9	53.6	18.5
5	3.0	53.6	17.9
6	3.8	11.6	3.1
8	2.8	91.8	32.8

[a]Due to an inefficient separation, the amounts of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine are not differentiated.

trate the formation of the nephrotoxic and mutagenic *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine in human urine after exposure to trichloroethene at the workplace.

Materials and Methods

Urine Collection

Urine was collected from individuals exposed to varying amounts of technical trichloroethene (purity not specified) during an 8-hr work shift when cleaning metal parts in a trichloroethene bath. Urine was collected for 16 hr after exposure and pooled.

Determination of Urinary Metabolites

Urinary trichloroacetic acid concentrations were determined according to the method of Tanaka and Ikeda (*6*). *N*-acetyl-*S*-dichlorovinyl-L-cysteine was quantified by GC–MS with selected ion monitoring (SIM) after a two-step concentration procedure. Briefly, the internal standard *N*-d_3-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (125 nmole) was added to 2 mL of the urine. The urine was then adjusted to pH 1 with concentrated HCl and extracted twice with ether. The ether extracts were evaporated and the residue further purified with a C18 column (Millipore). The obtained solution was dried and treated with borontrichloride/methanol for esterification. GC–MS was performed with a HP5890 gas chromatograph with mass selective detector (MSD) (*3*). For selected ion monitoring, the fragments m/z 144 and 147 were monitored during the gas chromatographic separation. For identification of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlovinyl)-L-cysteine, the pooled urine samples were adjusted to pH 1 with HCl and extracted with ether. The extract was then fractionated by high-performance liquid chromatography (Partisil ODS III, 80 × 250 mm, 5 μm, solvent A: TFA/H_2O, pH 2, B: methanol; A to B in 30 min, 3.0 mL/min).

Acylation of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine in Subcellular Fractions

N-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (0.2 μM) was incubated with kidney cytosol (3.4 mg protein/mL) for 60 min. At different time intervals, samples were taken and the concentrations of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2-dichlorovinyl)-L-cysteine in the samples were determined by high-performance liquid chromatography.

Table 2. Excretion of trichloroacetic acid and *N*-acetyl-*S*-dichlorovinyl-L-cysteine in the urine of Wistar rats and NMRI mice 22 hr after exposure (orally by gavage) to 1,1,2-trichloroethene (50 mg/kg).

Sex/species	*N*-acetyl-*S*-dichlorovinyl-L-cysteine, μmole/L[a]	Trichloroacetic acid, μmole/L	Trichloroacetic acid/mercapturic acid
Female rat	9.8	386	39.4
Male rat	10.1	222	22.0
Female mouse	4.0	290	72.5
Male mouse	14.5	222	15.3

Table 3. Deacetylation of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine measured in human kidney cytosol and kidney and liver cytosol of rats and mice.

Species	Formation of *S*-(1,2-dichlorovinyl)-L-cysteine, nmole/mg × min	
	Kidney cytosol	Liver cytosol
Human	0.41	ND
Wistar rat	0.35	0.08
F344 rat	0.61	0.11
NMRI mouse	0.94	0.17

ND, not determined.

Results and Discussion

Identification of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine

When a purified urine fraction was analyzed by GC–MS, two peaks with spectra and retention characteristics identical to those of synthetic *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine were present. These observations conclusively identify these toxic mercapturates as human trichloroethene metabolites (Fig.2).

Quantification of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine

By purification of urine and the use of the highly sensitive selected ion monitoring mode for GC–MS, the method used permitted the detection of 10 pmole of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine and the quantification of amounts between 50 and 10,000 nmole/L of human urine. The trideuterated mercapturic acid was used as internal standard to compensate for the loss of mercapturic acid during sample work-up. As shown in Table 1, humans exposed to trichloroethene excreted appreciable amounts of the two isomers of *N*-acetyl-*S*-dichlorovinyl-L-cysteine in urine. The SIM method used did not permit us to discriminate between *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine due to different separation conditions. Relative to the excreted amounts of trichloroacetic acid in mice and rat urine, human urine contained higher concentrations of the mercapturates (Tables 1 and 2).

Mercapturic acid biosynthesis and urinary excretion are multistep pathways that may be influenced by species-dependent differences in the activities of the individual enzymes involved. The concentration of mercapturates in urine may be dependent on the balance between deacetylation of the mercapturic acid and acetylation of the cysteine *S*-conjugates (*4*). Deacetylation

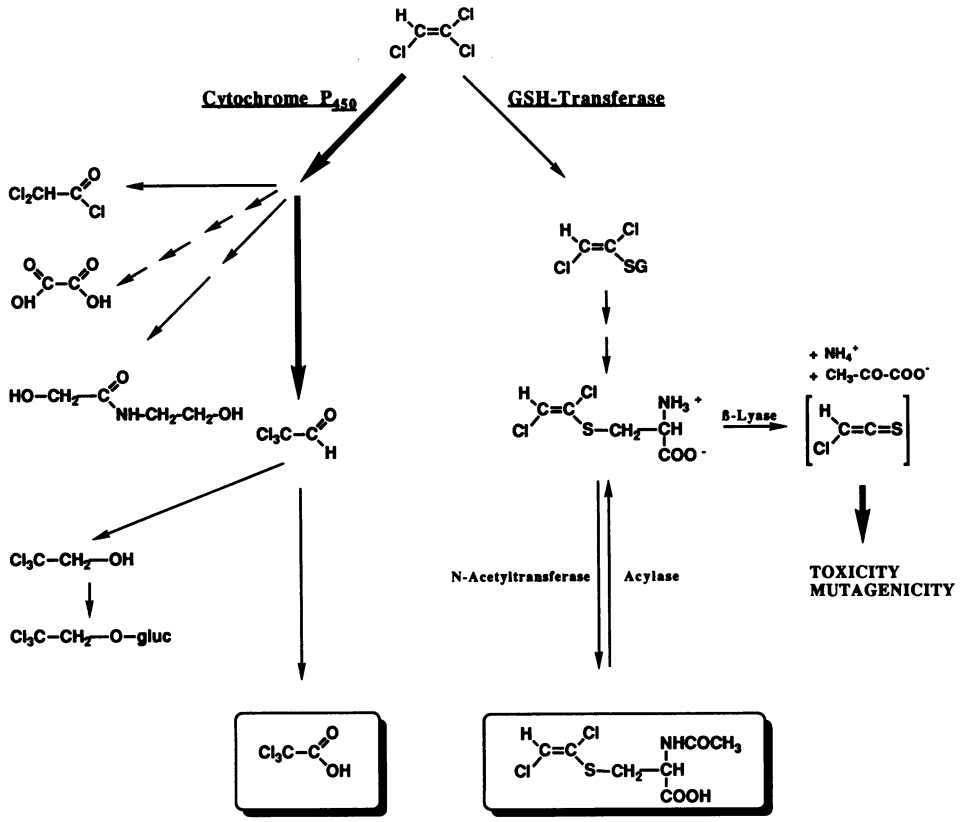

FIGURE 1. Metabolism of trichloroethene in rodents (*1*).

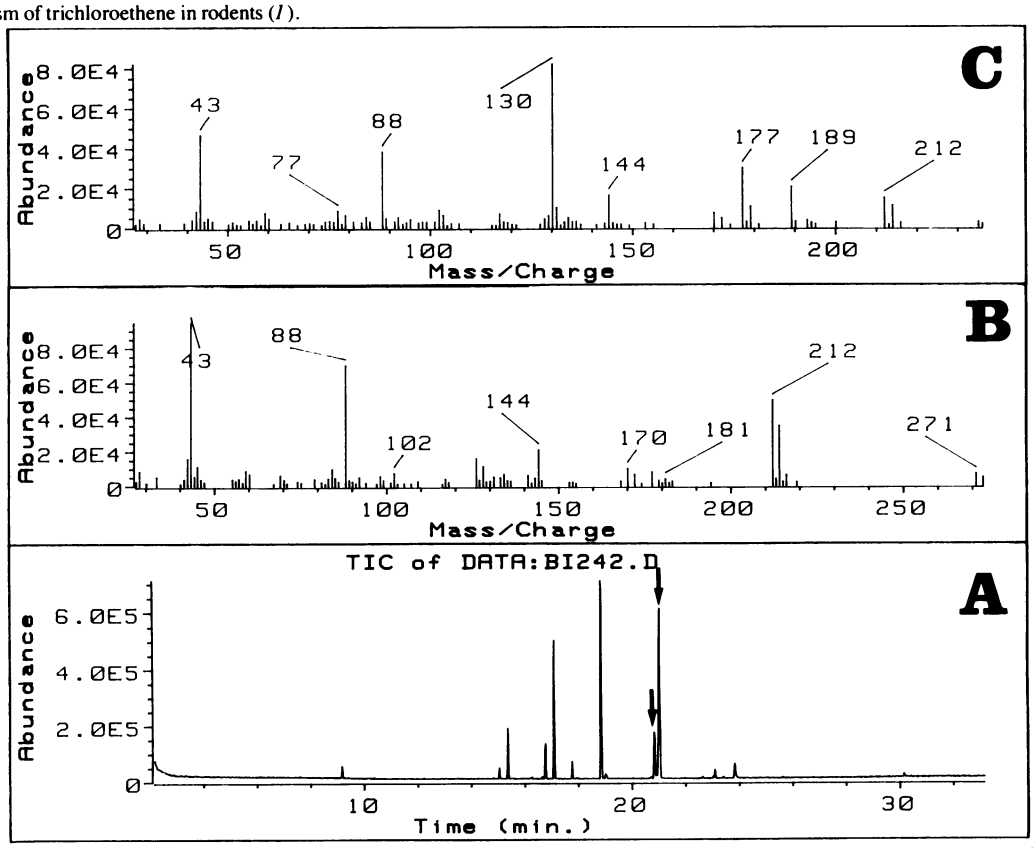

FIGURE 2. Gas chromatographic separation of a purified human urine sample (*A*) and mass spectra of *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine (*B*) and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (*C*) present in human urine after exposure to trichloroethene. Mass spectra were recorded in the electron impact mode with an ionization energy of 70 eV.

generates substrates for the final bioactivation step, the cysteine conjugate β-lyase-catalyzed reaction, in trichloroethene metabolism by glutathione conjugation. It may thus influence the excretion rates of mercapturic acids. We therefore studied the deacetylation of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine in kidney cytosol from different species (Table 3). Cytosol from NMRI mice showed the highest activity for *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine followed by the kidney cytosol from Fischer 344 rats. Human kidney cytosol and cytosol from the Wistar strain of rats revealed comparable rates, suggesting that the high excretion rates of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine in humans may be due to more intensive metabolism of trichloroethene by GSH conjugation in humans than in Wistar rats.

Our results demonstrate that *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine is a urinary metabolite of trichloroethene in occupationally exposed humans, which is excreted in substantial concentrations. The identification of this metabolite in human urine mandates a revision of the risk assessment of trichloroethene exposure. The precursor of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine, *S*-(1,2-dichlorovinyl)-L-cysteine, is a potent mutagen in the Ames test; its mutagenicity depends on bioactivation by β-lyase (*7*). Both *S*-dichlorovinyl-L-cysteine isomers are substrates for the β-lyase in rat kidney cytosol (Birner et al., unpublished data). Enzymes with β-lyase activity are present in human kidney, thus, formation of mutagenic metabolites is expected to occur in the kidney during trichloroethene metabolism. *S*-(1,2-dichlorovinyl)-L-cysteine is a weak inducer of DNA repair in mammalian kidney cells (*8*). In these cells it also induces cell proliferation, indicating the potential of this metabolite to exert both initiating and promoting activities in the renal tissue. Our results suggest that a mutagenic and nephrotoxic metabolite is formed in human trichloroethene metabolism and therefore a risk of nephrocarcinogenesis is associated with trichloroethene exposure.

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